

Communiqué

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Feature

Polyclonal Gammopathy - A Retrospective Cohort Study of 148 Patients

Inside

Ask Us

Abstracts of Interest

Calendar

Test Updates:

- Allergen Testing Specimens
- Copper and Zinc Method Change
- Free Insulin Test Code Change
- Hepatitis C Virus Detection by In Situ Hybridization Ordering Change
- Mumps IgG and IgM Method Change
- Urine Specimen Reminder for Protein Tests

New Test Announcements

Uniparental Disomy (UPD)

Polyclonal Gammopathy - A Retrospective Cohort Study of 148 Patients

Hypergammaglobulinemia results from an overproduction of immunoglobulins by plasma cells. This increased synthesis may be from several plasma lines (ie, polyclonal gammopathy), or from a single plasma cell clone (ie, monoclonal gammopathy). (See Figure 1.) The latter designation includes monoclonal gammopathy of undetermined significance (MGUS) and several malignant diseases, including multiple myeloma, primary systemic amyloidosis, Waldenström macroglobulinemia, and other lymphoproliferative disorders. In contrast, polyclonal gammopathy represents diffuse activation of B cells and is associated with a heterogeneous group of nonmalignant conditions.

Both polyclonal and monoclonal gammopathies are identified by serum protein electrophoresis (SPEP). Additional laboratory testing is necessary to characterize monoclonal gammopathies ([Monoclonal Protein Study #81756](#)). Visual inspection of SPEP is sufficient to diagnose polyclonal hypergammaglobulinemia and monoclonal protein studies are not routinely indicated. Despite the fact that it is unnecessary to perform monoclonal protein studies on polyclonal hypergammaglobulinemia specimens, we found that many of the specimens received by Mayo Medical Laboratories (MML) for monoclonal protein studies are subsequently determined to be polyclonal.

Recently, a retrospective study was conducted at Mayo Clinic to analyze associated conditions in patients in whom a moderate to marked polyclonal gammopathy had been diagnosed by visual inspection of specimen patterns on SPEP. The objective of this study was to quantify the clinical conditions and laboratory values associated with moderate to marked polyclonal gammopathy. The information presented should help clarify the distinction between monoclonal gammopathy and polyclonal gammopathy.

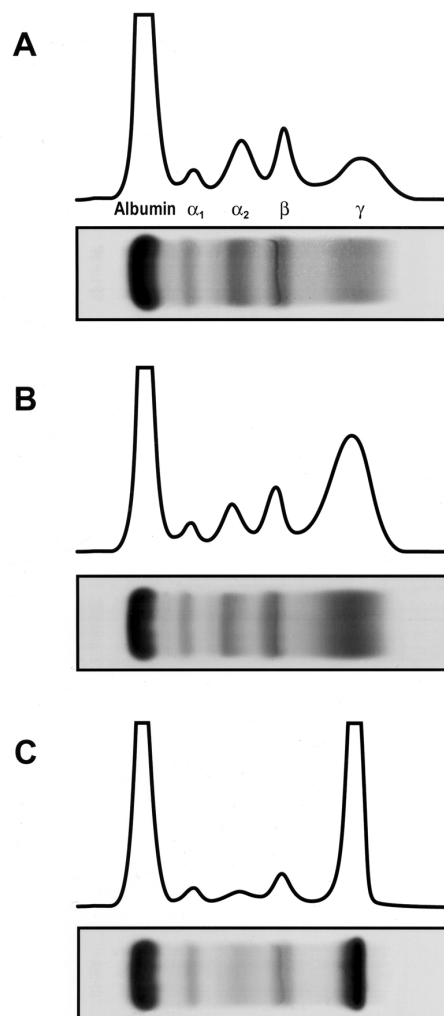


Figure 1. Electrophoretic patterns. A. Normal B. Polyclonal, broad outline comprising small peaks of many different proteins produced by many different plasma cells. C. Monoclonal, tall, narrow peak of homogeneous protein (single immunoglobulin), excessive output of single clone.

The study reviewed the medical records of all patients seen in a 1-year period at Mayo Clinic in whom a polyclonal gamma globulin level of 3.0 g/dL (normal = 0.7-1.7 g/dL) or higher had been identified (n = 148). A cutoff of 3.0 g/dL was chosen because this level of monoclonal hypergammaglobulinemia is frequently used to diagnose multiple myeloma. Patient characteristics are summarized in Table 1.

The diagnosis of polyclonal hypergammaglobulinemia was made by visual inspection of the SPEP patterns on cellulose acetate membranes. Immunoelectrophoresis and immunofixation studies were not performed in this cohort, nor were immunoglobulin isotype levels quantified. When available, albumin, aspartate aminotransferase, alkaline phosphatase, prothrombin time, creatinine, complete blood cell count with platelets, and differential results were abstracted.

Associated disease states were divided into 1 of 6 diagnostic categories—liver disease; connective tissue diseases (CTD), including vasculitides; hematologic disorders; nonhematologic malignancy; infectious disease (excluding viral hepatitis); and other. For the purposes of statistical analysis, the patients were divided into those with 1 diagnosis and those with more than 1 diagnosis. With the exception of 8 patients, diagnoses of associated disease predated first documented SPEP.

Laboratory Findings

Because many individuals in our cohort were established patients at Mayo Clinic, SPEP findings before and after the index SPEP existed for 65 and 75 patients, respectively. For those patients with more than 1 SPEP, there was variability of serum gamma globulin levels over time. Gamma globulin levels normalized in 16 patients, 9 after orthotopic liver transplants. In the

nontransplant patients, gamma globulin levels normalized within 3 to 5 years for 4 patients treated for autoimmune hepatitis. The 1 patient who had rheumatoid arthritis (RA) and large granular lymphocytic leukemia in 1991 had normal SPEP findings at 2 and 5 years. Another patient who had renal cell carcinoma was cancer free with a normalized gamma globulin level 18 months after her nephrectomy.

No difference in gamma globulin levels occurred between the 6 disease groups. Significant differences existed between groups for levels of aspartate aminotransferase, alkaline phosphatase, total bilirubin, albumin, erythrocyte sedimentation rate (ESR), creatinine, hemoglobin, and platelets.

Associated Diseases

In this study, 167 diagnoses were made in 148 patients with a polyclonal gammopathy, with 130 patients (88%) carrying only 1 diagnosis, including 5 (4%) with diagnoses that did not fit into the 5 classifications ("other").

Liver Disease

The existence of polyclonal gammopathy in patients with chronic liver disease is well recognized and represented the largest proportion of our cohort. The majority of patients had liver disease, including 79 patients (53%) with liver disease only and an additional 11 with other diseases. Several of these patients had more than 1 type of liver disease. More patients in the cohort with liver disease had autoimmune hepatitis (37%) than any other single diagnosis. Fifteen of 16 patients with virus-induced liver disease were infected with hepatitis C virus, 2 patients were coinfecting with both hepatitis B and hepatitis C virus, and 3 had concurrent alcohol-related hepatitides. Other liver disease diagnoses included primary biliary cirrhosis, primary sclerosing cholangitis, ethanol-induced liver

Table 1. *Clinical Characteristics*

Characteristic	All patients (n=148)	Patients with only 1 Diagnosis						Patients with >1 Diagnosis (n=18)
		Liver (n=79)	Connective Tissue Disease (n=28)	Hematologic Disorders (n=6)	Malignant (n=4)	Infection (n=8)	Other (n=5)	
Median age (y)	58	57	59	66	73	52	68	56
Range	15-86	16-84	15-83	48-80	66-76	31-80	34-73	28-86
Female sex (%)	59	51	86†	50	50	50	80	61
Hepatomegaly (%)	22	38	0	2	0	13	0	0
Splenomegaly (%)	26	34	0	33	0	25	0	0
Lymphadenopathy (%)	4.7	1.3	3.6	83	0	0	0	0

†P≤.01 vs liver group.

injury, cryptogenic cirrhosis, primary hemochromatosis, and α_1 -antitrypsin deficiency.

Our finding of a large percentage of hypergammaglobulinemic patients with autoimmune liver disease is consistent with previous reports. Elevation of serum gamma globulin levels is one of the distinguishing characteristics of autoimmune hepatitis, with more than 80% of these patients having hypergammaglobulinemia and with levels correlating with disease activity.

Connective Tissue Disease

The next most common group of diseases, occurring in 40 patients (27%), was CTD. Of these, 28 patients had CTD as their sole diagnosis and 12 had CTD with another disease. Of the 12 patients with CTD and more than 1 diagnosis, 7 had a concomitant hematologic disease, and 5 had liver disease. Sjögren syndrome was the most common diagnosis, followed by RA, systemic lupus erythematosus (SLE), and mixed CTD.

For patients with Sjögren syndrome, an association between anti-SS-A (Ro) positivity and the presence of hypergammaglobulinemia, purpura, leukopenia, anemia, and hypocomplementemia has been reported. In patients with RA and SLE, gamma globulin levels are elevated in as many as 37% and 58% of patients, respectively, and are reported to correlate with disease activity. Sjögren syndrome was overrepresented in our series relative to other rheumatic diseases (44% of all CTD diagnoses). Hypergammaglobulinemia has been reported to be an adverse prognostic sign for patients with ankylosing spondylitis.

Although the appearance of hypergammaglobulinemia in autoimmune rheumatic disease is not helpful in distinguishing one rheumatic disease from another, it may be central to the mechanism producing autoimmunity.

Hematologic Disorders

Hematologic disorders were the third most common associated diseases in our series, comprising 5% of all single diagnoses. Six patients had one of these hematologic disorders alone. An additional 7 patients had associated CTD. Patients had benign and malignant B and T phenotype lymphoproliferative disorders. In total, there were 2 benign lymphoproliferative disorders, 5 low-grade lymphomas, 2 high-grade T-cell lymphomas, 3 large granular lymphocytic leukemias, and 1 chronic myelomonocytic leukemia (a type of myelodysplastic syndrome). There were no cases of myeloma or primary systemic amyloidosis. In our series, the association of hematologic disease with

hypergammaglobulinemia was limited to lymphoproliferative disorders and 1 myelodysplastic syndrome. A third of patients with all categories of myelodysplastic syndrome and two-thirds of those with chronic myelomonocytic leukemia specifically are reported to have polyclonal gammopathy. Other nonmalignant conditions may be also associated with polyclonal gammopathy, including idiopathic neutropenia, idiopathic thrombocytopenic purpura, severe hemophilia A, thalassemia major, sickle cell anemia, and Fanconi anemia.

Nonhematologic Malignancy

Nine patients had an associated nonhematologic malignancy, 4 had an isolated malignancy and 5 had more than 1 disorder (liver disease or CTD). Malignancies included hepatocellular carcinoma, ovarian carcinoma, squamous cell carcinoma of the lung, pancreatic adenocarcinoma, renal cell carcinoma, and chondrosarcoma. Hepatocellular and ovarian carcinoma were most common.

Infections

An infectious disease (excluding viral hepatitis) was the sole cause of polyclonal gammopathy in 8 patients (5.4%). Four patients were infected with human immunodeficiency virus (HIV), 3 of whom were also asymptomatic carriers of hepatitis B, hepatitis C, or both. With HIV infection, gamma globulin levels tend to parallel the course of adenopathy, with mildly increased levels of IgG on seroconversion, gradually increasing levels until the diagnosis of the acquired immunodeficiency syndrome (AIDS), and declining levels by approximately 8 to 16 months after the first AIDS-defining illness.

Other

Five of the 148 patients could not be categorized into 1 of the 5 associative categories. Interestingly, 2 of these 5 patients developed lymphoproliferative disorders during follow-up. Whether the lymphoproliferative disorders had existed but had not been detected at the time the polyclonal gammopathy was diagnosed cannot be determined.

Survival

Of the 143 (62.9%) patients for whom follow-up was available, 90 were alive with a median follow-up of 67 months since their index SPEP. Survival for each of the groups was, as follows: CTD, 89%; liver, 62%; infection, 57%; hematologic, 33%; and malignancy, 0%. Survival for patients with more than 1 diagnosis was 50%. (See Figure 2.) Factors predictive of survival are shown in Table 2. Gamma globulin levels were not shown to be predictive of survival.

Table 2. Factors Significantly Predictive of Survival

Factor	P value
Univariate analysis	
Age	<.001
Albumin	<.001
Sex	.009
Disease group	.002
Platelets	.003
Creatinine	.004
Multivariate analysis	
Age	<.001
Albumin	<.001
Disease group	.001
Platelets	.02

Treatment of Polyclonal Gammopathy

No specific therapy is indicated for polyclonal gammopathy. Treatment is directed at the underlying disease. There have been reports, however, of hyperviscosity syndrome associated with polyclonal gammopathy in RA, SLE, Sjögren syndrome, AIDS, chronic active hepatitis, pseudolymphoma, and Castleman disease. Symptomatic hyperviscosity secondary to polyclonal gammopathy has been effectively treated with plasmapheresis or corticosteroids.

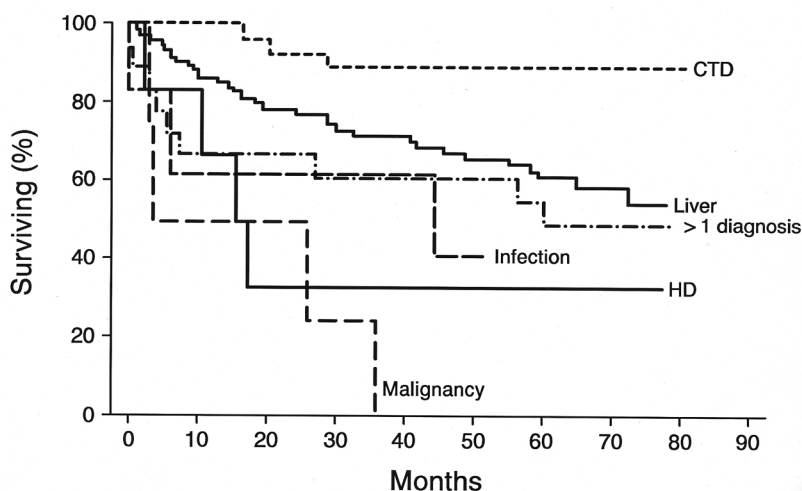
Conclusions

This study was performed to quantify clinical conditions and laboratory values associated with moderate to marked polyclonal gammopathy. Of the 148 patients in the study, 130 patients (88%), had only 1 diagnosis. Liver disease was the most common single disease association in 79 (61%) of these 130 patients, followed by connective tissue diseases in 28 (22%), chronic infections in 8 (6%), hematologic disorders in 6 (5%), and nonhematologic malignancies in 4 (3%). No difference in gamma globulin levels existed between groups. By multivariate analysis, age, albumin concentration, disease group, and platelet count were predictive of survival. No patient developed myeloma or a clonal plasmaproliferative disorder.

Moderate to marked polyclonal gammopathy may reflect an underlying condition: liver disease, CTD, hematologic disorder, infection, or malignancy.

Adapted from Mayo Clinic Proceedings 76:476-487, 2001. References and 2 tables omitted.

Figure 2. Survival by disease group. CTD = connective tissue disease; HD = hematologic disorder.



Abstracts of Interest

Fatal Sepsis in a Patient With Rheumatoid Arthritis Treated With Etanercept

Mercedeh Baghai, MD; Douglas R. Osmon, MD, MPH; Donna M. Wolk, PhD; Lester E. Wold, MD; George J. Haidukewych, MD; And Eric L. Matteson, MD, MPH

Patients with long-standing, severe, erosive rheumatoid arthritis who have extra-articular manifestations and have undergone joint replacement surgery are at increased risk for serious infection and premature mortality. New therapies, including cytokine antagonists, hold great promise for improving the course of rheumatoid arthritis. However, they have powerful anti-inflammatory effects that may mask symptoms of serious infection. We report a case of fatal pneumococcal sepsis occurring in a 37-year-old woman with rheumatoid arthritis treated with the tumor necrosis factor antagonist etanercept and suggest management strategies for early detection and management of this complication.

Mayo Clinic Proceedings 2001;76:653-656

Allergen Testing Specimens

MML offers allergen testing of over 400 individual allergens. When individual allergen testing is performed, the laboratory can test 5 or more individual allergens from a single 1.0 mL serum specimen. Allergen testing of more than 5 individual allergens for the same patient requires additional specimen volume, but **does not require a separate specimen**. It is unnecessary, and undesirable, to submit separate specimens for individual allergens.

The standard specimen tube for allergen testing is the 13- x 75-mm tube. The contents of 1 tube can be used to test a **minimum** of 20 allergens. When submitting specimens for individual allergen testing, it is best to submit 1 tube with a larger volume of specimen, rather than 2 or more tubes.

Copper and Zinc Method Change

The method has changed for both [Zinc, Urine #8591](#) and [Copper, Urine #8590](#). Previously, these tests were performed as an inductively coupled plasma emission (ICP) assay. The new method utilizes inductively coupled plasma emission and mass spectrometry (ICP-MS). Comparisons performed in our laboratory demonstrated improved precision and sensitivity with the new method. This change does not affect reference values, analytic time, or specimen requirements.

Free Insulin Test Code Change

Effective February 6, 2002, the test code for [Insulin, Free, Serum](#) will change. The test code change will facilitate ordering; there are no changes to the method, reference values, or specimen requirements.

New Test Code

81728

Previous Test Code

320

Hepatitis C Virus Detection by *In Situ* Hybridization Ordering Change

[Hepatitis C Virus \(HCV\) Detection by *In Situ* Hybridization #81965](#) is no longer orderable as a single stain. When this test is requested, a [Surgical Pathology Consultation #5439](#) will be added and charged.

For greatest efficiency, it is recommended that you order [Surgical Pathology Consultation #5439](#) and indicate hepatitis C virus stain on the "Pathology/Cytology *In Situ* Request Form" (Supply T246). This form is required for processing your specimen

Urine Specimen Reminder for Protein Tests

Recent changes to [Electrophoresis, Protein, Urine #82441](#) and [Monoclonal Protein Study, Urine #8823](#) resulted in changes to the specimen required for these 2 profiles. Each of these tests require 30 mL from a 24-hour urine collection, with no preservative added. Transfer the 30 mL into 3 plastic, 13 mL urine tubes, package together and send refrigerated to MML.

Mumps IgG Method Change

The method for the IgG portion of [Mumps Virus Antibody, IgG and IgM \(Separate Determinations\), Serum #8761](#), was changed from an indirect immunofluorescence assay to an enzyme-linked fluorescent immunoassay (EIA). The IgM portion of the test remains an indirect immunofluorescence assay. The method change has resulted in a change to the reference values.

New Reference Values

IgG: negative

IgM: negative

Previous Reference Values

IgG: <1:5

IgM: <1:10

Meeting Calendar

Interactive Satellite Programs . . .

March 12, 2002

The Prevention & Management of Type 2 Diabetes
Presenter: Mehmood Khan, MD - Moderator: Robert Kisabeth, MD

April 23, 2002

Complementary & Alternative Medicine: From Traveling Medicine Shows to the Internet and the NIH
Presenter: Brent Bauer, MD - Moderator: Robert Kisabeth, MD

September 17, 2002

Advances in Wound Healing
Presenter: Steve Kavros, DPM - Moderator: Robert Kisabeth, MD

October 22, 2002

Bone Marker Assays: Are They Useful for the Diagnosis & Treatment of Osteoporosis?
Presenter: Lorraine Fitzpatrick, MD - Moderator: Robert Kisabeth, MD

November 19, 2002

HIV Update
Presenter: Zelalem Temesgen, MD - Moderator: Robert Kisabeth, MD

December 10, 2002

Stroke Prevention and Management
Presenter: David Wiebers, MD - Moderator: Robert Kisabeth, MD

Upcoming Education Conferences . . .

Practical Spirometry

March 12-13, 2002

Mayo Clinic, Siebens Building
Rochester, Minnesota

September 13-14, 2002

Holiday Inn Chicago City Centre
Chicago, Illinois

October 31-November 1, 2002

Mayo Clinic, Siebens Building
Rochester, Minnesota

Course Director: Paul D Scanlon, MD
Presented by Mayo Pulmonary Services

Integration Through Community Laboratory Insourcing: From Mission Statement to Successful Implementation

May 1-3, 2002

Hilton in the WALT DISNEY WORLD, Resort
Lake Buena Vista, Florida
Course Director: Rodney Forsman



For a complete listing of all the courses offered throughout the year, contact the Mayo Reference Services
Education Office at 1-800-533-1710 or 507-284-8742.



Q: MML offers a DNA test for the genes most commonly responsible for hereditary hemochromatosis (HH). When is that test indicated?

A: The test, [Hereditary Hemochromatosis Gene Analysis, Blood #81508](#), is indicated in two circumstances:

- 1) To support the diagnosis of HH in a patient who is strongly suspected of having this disorder on the basis of other findings. These indications are further discussed below.
- 2) To identify siblings and other first-degree adult relatives at high risk of HH in a family in which one member has been found to have HH in association with the mutant genes.

HH is an autosomal recessive disorder of iron metabolism that is characterized by an accelerated rate of intestinal iron absorption and progressive iron deposition in various tissues. The consequences of this iron overload are extremely variable, with multiple systems often being involved. Many cases are asymptomatic. The most common symptoms are fatigue, impotence, arthralgias, abdominal pain, and cardiac palpitations. Late symptoms are those of organ failure, such as those attendant on heart failure, cirrhosis of the liver, hepatic cancer, hypothyroidism, hypogonadism, diabetes mellitus, hyperpigmentation, and arthritis.

Persons at risk for HH typically have elevated serum transferrin saturation, >45%, from an early age, which may be considered the "hallmark" of HH. (Serum transferrin saturation is calculated, in %, from $100 \times \text{serum iron conc.} / \text{divided by serum iron binding capacity}$). As iron overload develops, they also have elevated serum ferritin concentrations, typically >500 µg/L. For patients who are clinically suspected of having HH without known family history, the DNA test is often indicated if the serum transferrin saturation is >45% on at least two occasions and the serum ferritin concentration is also elevated. (Note: It is critical that patients not be taking iron-containing medication, including over-the-counter multiple vitamins with iron, for at least a week prior to tests for serum transferrin saturation, as ingested iron will almost always elevate the transferrin saturation and may lead to diagnostic errors. Further, elevated serum iron (iron, iron binding capacity, and transferrin saturation) tests, if collected in the non-fasting state, should be confirmed on a second fasting specimen. Hormonal contraceptives may also spuriously elevate transferrin saturation.) For patients who have evidence of liver injury, or serum ferritin >1000 µg/L, a liver biopsy is usually warranted. The DNA test for HH is not a substitute for liver biopsy.

The gene responsible for HH (HFE) is located on chromosome 6 and is closely linked to the human leukocyte antigen complex (6p21.3). The majority of HH patients have mutations in this HFE gene. The HFE gene is very common in Caucasians of European origin: approximately 1 out of every 10 persons are carriers, having a deleterious mutation on one chromosome 6; approximately 1 out of every 250-500 persons are homozygotes, having a mutation on both chromosomes 6.

The most deleterious mutation is at nucleotide 845 (G→A). This G→A mutation corresponds to the amino acid substitution of tyrosine for cysteine at amino acid position 282 in the HFE protein (therefore, this mutation has been designated C282Y, C for cysteine, Y for tyrosine). In a 1999 study, 85% of patients with iron overload were found to be homozygous for the C282Y mutation. (Brandhagen, et al. *Am J Gastroenterol* 1995;(10):2910-2914)

Most cases of HH (approximately 80%) are homozygotes, having the C282Y mutation on both chromosomes 6. Recent studies indicate that less than half, and perhaps less than 10%, of homozygotes ever develop severe disease. Heterozygotes, who are carriers with only one affected chromosome 6, have very little risk of ever developing hemochromatosis. Family studies are important, because within an affected sibship, approximately one-fourth of the siblings will be homozygotes and at increased risk of developing iron overload. Thus, when one

continued on page 8

case is identified within a family, it is appropriate to test to see whether there are other siblings who will be similarly affected.

Two other *HFE* mutations may contribute to iron overload. The more common of these is at nucleotide 187 (C→G). In the *HFE* protein, this mutation corresponds to a substitution of aspartic acid for histidine at amino acid position 63 (therefore, this mutation has been designated H63D, H for histidine, D for aspartic acid). Approximately 20-25% of whites are carriers of this mutation and 2% are homozygous. This mutation appears to enhance iron absorption slightly. Homozygous H63D is rarely associated with iron overload. Heterozygous carriers are not affected. However, some persons with moderate iron overload are positive for both C282Y and for H63D; ie, they are compound heterozygotes.

[Hereditary Hemochromatosis Gene Analysis, Blood #81508](#), can be used for confirmation of a clinical diagnosis of HH, testing of asymptomatic individuals with increased blood test of iron stores (increased percent saturation of transferrin or serum ferritin), and predictive testing of individuals who have a family history of HH. MML recommends careful consideration of the advantages and disadvantages of genetic testing – genetic counseling is recommended before pursuing predictive testing for genetic disorders. Liver biopsy remains an important tool for diagnosis and for predicting prognosis of this disease.

Caution: Although the DNA test is useful in the circumstances given above, it is important to recognize that more than 10% of HH patients, including those with severe disease, do not have the presently known *HFE* mutations nor any other HH-related mutations on chromosome 6. Further studies are underway to attempt to explain these cases. **Negative tests for the HH genes do not rule out HH.**

Communiqué

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The Communiqué is published by Mayo Reference Services to provide laboratorians with information on new diagnostic tests, changes in procedures or normal values, and continuing medical education programs and workshops.

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